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# Spectrophotometric Method for the Estimation of Total Alkaloids in the Stem Bark of *Symplocos racemosa* and in Its Formulations

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A simple sensitive, precise, reproducible and accurate UV/ Visible spectrophotometric method of estimation of alkaloids in stem bark of *Symplocos racemosa* (Lodhra) and in its formulations using tropaeolin 'OO' was developed. The method was found suitable for alkaloids bound to tannins causing interference in the analysis process. The method can be adopted as routine tool for standardization and quality control.

**Key Words: *Symplocos racemosa*, Spectrophotometery, Total alkaloids.**

## INTRODUCTION

Stem bark of *Symplocos racemosa* Roxb, (Symplocaceae), known as Lodhra in Ayurveda, is an ingredient of many rasayanas. The bark is astringent and therefore used to treat diarrhoea and dysentery. It is used as tonic, antioxytocic, amoebicide and in conjunctivitis and opthalmia[1,2]. A decoction of bark is used to treat bleeding gums, mennorrhagia and other uterine disorders[3]. Symposide a glycoside showed antifibrinolytic activity, alcoholic extract reduced the frequency and intensity of contractions *in vitro* in both pregnant and non-pregnant uteri. Antagonisation of acetylcholine-induced contractions and prolongation of the quiescent period were also observed[4]. The therapeutic efficiency of *S. racemosa* is due to

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presence of alkaloids, of which, harmine is the major one[5]. In the present investigation, alkaloids from *Symplocos racemosa* stem bark and the market formulations containing lodhra were estimated by UV/Visible spectrophotometric method using tropaeolin 'OO'. This method has applicability in the routine quality control for estimation of total alkaloids in both stem bark of *S. racemosa* and its formulations.

## EXPERIMENTAL

Two different samples of stem bark of *Symplocos racemosa* from different geographical sources were collected as follows:
Sample1 (**S1**) : Stem bark sample from Bangalore.
Sample2 (**S2**) : Stem bark sample from Chittoor.

The samples were authenticated in by comparing with standard samples kept in the Pharmacognosy laboratory and voucher specimens were preserved. Samples of marketed formulation containing *S. racemosa* (M1 and M2) were purchased from the local market.

Harmine (98 %) as chemical marker was purchased from Himedia, Mumbai, India. All the chemicals used were of analytical grade. Reagents used include acetate buffer of pH 4.6 (5.4 g sodium acetate and 2.66 mL glacial acetic acid in 100 mL of double distilled water), tropaeolin 'OO' solution (standard solution of tropaeolin 'OO' in double distilled water) and acid reagent (1 % v/v sulfuric acid in methanol).

**Procedure for extraction of alkaloids:** Method described in European Pharmacopoeia[6] was adopted, for the extraction of alkaloids from the samples (1 g of stem bark/1 g of powdered 20 tablets of marketed formulation). The method was subjected to modification by replacing 10 % NaOH solution with 25 % ammonia and 3 g tragacanth with 2 g of carboxymethoxy cellulose sodium salt.

Suitable quantities of sample solution were taken and colour was developed as described above. Absorbance of coloured complex was recorded at 545 nm. The amount of total alkaloids from sample solution were calculated from the calibration curve and represented as harmine.

**Calibration curve:** Different aliquots of 0.25-2.00 mL standard solution of harmine (100 µg/mL) in methanol were taken and subjected to analysis as per method of Haussler[7]. Calibration curve was prepared by plotting concentration of harmine *vs.* absorbance. The colour developed was measured at 545 nm against blank, using double beam UV-Vis spectrophotometer (Shimadzu 1601).

**Estimation of total alkaloids in the stem bark and formulation of *S. racemosa*:** Suitable aliquots of sample solutions were taken for the estimation of total alkaloids in stem bark and in the marketed formulations. Absorbance of the coloured solution developed was recorded at 545

nm. The amount of total alkaloids in the stem bark and marketed formulations was calculated using calibration curve. The content of the total alkaloids in the different samples was expressed in terms of harmine.

**Method validation:** The method of analysis was validated for precision by repeating the experiment 5 times with the same quantity of harmine. The accuracy of the method was determined by performing the recovery study at two levels, by adding known amount of harmine to the stem bark sample.

## RESULTS AND DISCUSSION

The method is based on the reaction between alkaloids and tropaeolin 'OO' to form a charge transfer complex, which can be extracted in chloroform or dichloromethane, followed by its reaction with acid reagent to give a purple coloured chromogen detectable at wavelength maxima of 545 nm[8-10]. Various parameters are to be optimized affect the development of colour like the pH and amount of acetate buffer used, amount of tropaeolin 'OO' solution required, amount of acid reagent consumed and time to complete the reaction. The chromogen formed was found to be stable up to 3 h. The calibration curve for harmine was found to be linear over the range of 0.5-4.0 µg/mL with a correlation coefficient of 0.997. Precision of the method, expressed as relative standard deviation, was found to be 1.02% (Table-1) and the average percentage recovery was 98.11 % (Table-2). The method was applied for the estimation of total alkaloids in the stem bark of *S. racemosa* and in two market formulations. The results of the analysis are shown in (Table-3).

TABLE-1
METHOD VALIDATION PARAMETERS

| Parameters | Results | Parameters | Results |
|---|---|---|---|
| Linearity range (µg/mL) | 0.5-4.0 | Intercept | -0.00582 |
| Correlation coefficient | 0.997 | Precision (n = 5, RSD %) | ± 1.02 |
| Slope | 0.003874 | Accuracy (%) | 99.1 |

TABLE-2
PERCENT RECOVERY STUDIES*

| Amount of total alkaloids present (µg) | Amount of harmine added (µg) | Amount of total alkaloids found (µg) | Recovery (%) |
|---|---|---|---|
| 99 | 50 | 146.4 ± 3.47 | 98.27 ± 2.32 |
| 99 | 100 | 194.9 ± 3.63 | 97.96 ± 1.82 |

*Mean ± standard deviation; n = 3

TABLE-3
CONTENT OF TOTAL ALKALOIDS IN THE STEM BARK OF *S. racemosa* AND MARKETED FORMULATIONS

| Samples | Content of total Alkaloids* (% w/v) | Samples | Content of total Alkaloids* (% w/v) |
|---|---|---|---|
| Stem bark | | Marketed formulation | |
| S1 | 2.13 ± 0.168 | M1 | 0.98 ± 0.012 |
| S2 | 3.02 ± 0.087 | M2 | 0.66 ± 0.023 |

*Mean ± standard deviation; n = 3
Content of total alkaloids in the stem bark of *S. racemosa* and marketed formulations
S1 = Stem bark of *Symplocos racemosa* from Bangalore
S2 = Stem bark of *Symplocos racemosa* from Chittoor
M1 = Market formulation containing *Symplocos racemosa*
M2 = Market formulation containing *Symplocos racemosa*

In conclusion, the proposed method is simple, sensitive, precise and accurate and can be used as a part of routine quality control of stem bark of *S. racemosa* as well as to assess the labeled amount of drug added in the formulations. Since other alkaloids also form coloured complex with tropaeolin 'OO'[11-13]. The limitation of the present method is its specific for the alkaloids of *S. racemosa* and therefore is applicable only to those formulations which do not contain any other alkaloid containing drug along with *S. racemosa*.

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